

What is claimed:

1. An isolated nucleic acid molecule comprising:
  - (a) nucleotide sequences encoding a bacteriophage recombinase function;
  - 5 (b) nucleotide sequences encoding a bacteriophage anti-recombinase function;
  - (c) *Ptac* promoter sequences operably linked to the nucleotide sequences of (a) and (b); and
  - (d) nucleotide sequences encoding LacI operably linked to its native  
10 promoter.
2. The nucleic acid molecule of claim 1, further comprising origin of replication sequences which confer low copy number on a vector comprising the nucleic acid molecule.  
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3. The nucleic acid molecule of claim 2, wherein the origin of replication is temperature sensitive.
4. An isolated nucleic acid molecule comprising:
  - 20 (a) nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase function;
  - (b) nucleotide sequences encoding bacteriophage  $\lambda$  anti-RecBCD function;
  - (c) *Ptac* promoter sequences operably linked to the nucleotide sequences of (a) and (b); and
  - 25 (d) nucleotide sequences encoding LacI operably linked to its native promoter.
5. The nucleic acid molecule of claim 4, further comprising origin of replication sequences which confer low copy number on a vector comprising the nucleic  
30 acid molecule.
6. The nucleic acid molecule of claim 5, wherein the origin of replication is temperature sensitive.

7. The nucleic acid molecule of any one of claims 4-6, wherein the nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase function comprise  $\lambda$  *exo* and *bet* sequences.

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8. The nucleic acid molecule of any one of claims 4-6, wherein the nucleotide sequences encoding  $\lambda$  anti-RecBCD function comprise  $\lambda$  *gam* sequences.

9. A vector comprising:

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(a) nucleotide sequences encoding a bacteriophage recombinase function;

(b) nucleotide sequences encoding a bacteriophage anti-recombinase function;

(c) *Ptac* promoter sequences operably linked to the nucleotide sequences of (a) and (b);

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(d) nucleotide sequences encoding LacI operably linked to its native promoter; and

(e) origin of replication sequences which confer low copy number on the vector.

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10. The vector of claim 9, wherein the origin of replication sequences are temperature sensitive.

11. A vector comprising:

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(a) nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase function;

(b) nucleotide sequences encoding bacteriophage  $\lambda$  anti-RecBCD function;

(c) *Ptac* promoter sequences operably linked to the nucleotide sequences of (a) and (b); and

(d) nucleotide sequences encoding LacI; and

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(e) origin of replication sequences which confer low copy number on the vector.

12. The vector of claim 11, wherein the origin of replication sequences are temperature sensitive.

13. The vector of claim 12, wherein the nucleotide sequences encoding  
5 bacteriophage  $\lambda$  Red recombinase function comprise  $\lambda$  *exo* and *bet* sequences.

14. The vector of claim 12, wherein the nucleotide sequences encoding  $\lambda$  anti-RecBCD function comprise  $\lambda$  *gam* sequences.

10 15. A recombinant organism comprising the vector of any one of claims 9-14.

16. The recombinant organism of claim 15, which is a bacteria.

15 17. The recombinant organism of claim 16 which is of the genus *Escherichia*.

18. The recombinant organism of claim 17, which is *Escherichia coli*.

20 19. The recombinant organism of claim 18, which is *Escherichia coli* K12.

20. The recombinant organism of claim 16 which is a pathogenic species.

21. The recombinant organism of claim 20 which is a pathogenic *Escherichia*  
25 *coli*.

22. The recombinant organism of claim 21 which is enterohemorrhagic *E. coli* (EHEC) or enteropathogenic *E. coli* (EPEC).

30 23. The recombinant organism of claim 15 which is of the genus *Pseudomonas*.

24. The recombinant organism of claim 23, which is *Pseudomonas aeruginosa*.

25. The recombinant organism of claim 15 which is of the genus  
5 *Mycobacterium*.

26. The recombinant organism of claim 25, which is *Mycobacterium tuberculosis*.

10 27. A method of promoting efficient recombination of genetic material in a microorganism comprising use of the vector of any one of claims 9-14.

28. The method of claim 27, wherein the genetic material is endogenous.

15 29. The method of claim 27, wherein the genetic material is exogenous

30. The method of claim 27, wherein the genetic material is derived from a prokaryote.

20 31. The method of claim 27, wherein the genetic material is derived from a eukaryote.

32. The method of claim 27, wherein the genetic material is derived from a fungi.

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33. A method for determining whether a bacterial gene is a potential drug target comprising:

(a) introducing a test construct into the microorganism of claim 15, wherein the test construct comprises an integrating segment flanked by recombination segments;  
30 wherein the recombination segments are homologous to the bacterial gene or surrounding sequences; and

(b) culturing the microorganism under conditions such that recombination between the test construct and the bacterial gene occurs; and

(c) assaying the microorganism for growth and/or pathogenicity or an indicator thereof,

whereby a change in growth and/or pathogenicity or an indicator thereof identifies the bacterial gene as a potential drug target.

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34. The method of claim 33, wherein the bacterial gene is chromosomal.

35. The method of claim 33, wherein the bacterial gene is present on an endogenous plasmid.

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36. The method of claim 33, wherein the integrating segment comprises nucleotide sequences encoding a selectable marker.

37. The method of claim 36, wherein the selectable marker is selected from the group consisting of ampicillin (Amp), kanamycin (Kan), tetracycline (Tat), and  $\beta$ -glycosidase ( $\beta$ -gal).

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38. A method of cloning a potential vaccine antigen comprising:

(a) introducing a substrate into the microorganism of claim 15, wherein the substrate comprises recombination segments comprising nucleotide sequences homologous to a potential vaccine antigen gene or surrounding native sequences; and  
(b) culturing the microorganism under conditions such that recombination between the substrate and the vaccine-antigen gene sequences or surrounding native sequences occurs;

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25 such that *in vivo* cloning of the vaccine antigen occurs.

39. A vaccine comprising an antigen identified according to the method of claim 38.

40. Use of the recombinant organism of claim 20 in the manufacture of a vaccine.

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41. A method of producing an attenuated pathogenic microorganism, comprising:

(a) introducing a vector of any one of claims 9-14 into a pathogenic microorganism;

5 (b) introducing a substrate into the pathogenic microorganism, wherein the substrate comprises recombination segments comprising nucleotide sequences homologous to a gene required for pathogenicity or surrounding native sequences; and

(c) culturing the microorganism under conditions such that  
10 recombination between the substrate and the gene sequences or surrounding native sequences occurs;

such that the gene required for pathogenicity is mutated, thereby producing an attenuated pathogenic microorganism.

15 42. An attenuated pathogenic microorganism produced according to the method of claim 41.

43. A vaccine comprising an attenuated pathogenic microorganism of claim  
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